II. REMARKS

This Amendment is being submitted in response to the final Office Action dated June 1, 2009 in the above-identified application. A Notice of Appeal was filed in this application on December 1, 2009. Concurrently with this Amendment, Applicant submits a petition for a five month extension of time to file an Appeal Brief, along with the requisite fee. Therefore the time for filing an Appeal Brief is extended from February 1, 2010 to July 1, 2010, and this Amendment is being timely filed. Applicant also files concurrently with the present Amendment a Request for Continued Examination, along with the requisite fee. If it is determined that any additional fee is due in connection with this filing, the Commissioner is authorized to charge said fees to Deposit Account No. 50-0552.

A. Status of the Claims

Claims 1, 2, 4-9, 12, 13 and 15-19 are canceled without prejudice by virtue of the present Amendment and new claims 43 – 64 are added. Claims 43-64 are the only claims currently pending. Support for these claims are found throughout the specification as filed.

New claims 43-58 are supported by the description on at least page 3, lines 22-25, page 6, lines 5-23, page 7, line 26 to page 8, line 3 and in Example 5. That is, the description teaches methods of analysing and/or removing contaminating nucleic acids by comparing the methylation status of contaminating and non-contaminating nucleic acids (i.e. target DNA). The description teaches one method for comparing the methylation status of contaminating nucleic acids and target DNA, that is, by treating the sample with bisulfite, as exemplified in Example 5.

A method described in detail in the specification is the result of the chemical modification of the cytosine in contaminating nucleic acids, such as amplicons, which is converted to uracil by the

bisulfite treatment. In contrast, methylated cytosine in genomic DNA is not converted to uracil. We refer to page 26, line 24 to page 27, line 13 of the present specification where this feature is explained in detail. As such, bisulfite treatment will preferentially chemical modify the contaminating nucleic acids thereby rendering them removable from a sample. Furthermore, such chemical treatment will also provide the opportunity to compare the methylation status of the contaminating nucleic acid with 'target' DNA so that the sample may be analyzed for the contaminating DNA and also the presence of the target DNA itself due to the difference in methylation status.

Support for new claims 59 to 63 may be found on at least page 6, line 5 to page 7, line 22. Support for new claim 64 may be found on at least page 9, lines 7-22.

It is submitted that no new matter has been added by virtue of this amendment

Reconsideration of the application is respectfully requested.

B. Claim Rejections- 35 U.S.C. § 112

Claim 12 was rejected under 35 U.S.C. § 112, second paragraph as being indefinite over the recitation of "which further comprises PCR.." Claim 12 has been canceled and therefore this rejection is moot.

Claims 13 and 15-18 were rejected under 35 U.S.C. § 112, second paragraph as being indefinite over the recitation of "the pre-treatment step of removing cell bound contaminating nucleic acids." Claim 13 and 15-18 have been canceled and therefore this rejection is moot.

Claim 19 was rejected under 35 U.S.C. § 112, second paragraph as being indefinite over the recitation of "the pre-treatment steps comprise removing cell bound contaminating nucleic acids from the sample by exposing nucleic acids in the cells and then removing the nucleic acids" as lacking antecedent basis. Claim 19 has been canceled and therefore this rejection is moot.

C. Claim Rejections under 35 U.S.C. § 102

Rejection under 35 U.S.C. § 102(b) - Walker (EP 0585660)

Claims 1, 2, 4-9 and 12 stand rejected under 35 U.S.C. § 102(b) as anticipated by Walker (EP 0585660). Claims 1, 2, 4-9 and 12 have been canceled in the present amendment, and therefore the rejection of claims 7 and 8 is moot. Applicants believes that new claims 43-64 are not anticipated by the Walker patent as set forth below.

New Independent Claims 43, 50, 59 and 61 recite:

> 43. A method of removing contaminating nucleic acids from a sample and then analyzing the sample comprising the steps of chemically treating the sample, wherein the chemical treatment results in the chemical modification of the contaminating nucleic acid and then analyzing the sample.
>
> 50. A method of analyzing a nucleic acid sample for genomic DNA comprising the steps of
>
> (i) chemically treating the sample, wherein the chemical treatment results in the chemical modification of contaminating nucleic acid;
>
> (ii) treating the sample with an enzyme; and

(iii) characterizing the treated sample for the genomic DNA.

59. A method of removing contaminating nucleic acids from a sample and then analyzing the sample comprising the step of enzymatically treating the sample, wherein the enzymatic treatment results in the inactivation of the contaminating nucleic acid, and then analyzing the sample.

61. A method of removing contaminating nucleic acids from a sample and then analyzing the sample comprising the steps of physically treating the sample, wherein the physical treatment results in the removal of the contaminating nucleic acid, and then analyzing the sample.

The chemical, enzymatic or physical treatment of the presently claimed invention is a step to remove the contamination from the sample. After that the sample can be used in methods to analyse the sample and thereby detect genomic DNA, knowing that the sample is not contaminated.

The invention can remove the contaminating nucleic acid by chemical modification of the contaminating nucleic acid, leaving behind a sample that can be used to identify the genomic DNA using known techniques. The basis of chemical modification as shown in Example 5 is the chemical will only convert unmethylated-C to uracil. In contrast, much of the genomic DNA comprises methylated-C, which would not be converted to uracil.

The invention also provides a method for comparing the methylation status of cytosine in a sample. For example, a sample of genomic DNA would have a higher amount of methylated-C than a sample contaminated with nucleic acids derived from amplicons. The calculation of such a ratio would occur after the sample was treated with the chemical.

The use of enzymes or probes also relies on the difference in methylation of the genomic DNA versus the contaminating nucleic acids.

In contrast, the Walker patent describes a method of decontaminating the products of a nucleic acid amplification reaction (amplicons) from a nucleic acid sample with exonucleases. See Walker, paragraph [0001]. The Walker patent is directed towards the use of probes in a method of identifying contamination, which is not the focus of the presently amended claims.

The Walker patent teaches an amplicon decontamination method comprising combining a nucleic acid preparation containing target nucleic acid (which may be contaminated with amplicons [contaminating nucleic acids]) with a single strand specific exonuclease. The mixture is then incubated for a sufficient time to degrade the amplicons. This is clearly set out on page 2, lines 24-30 of the document, under "Summary of the Invention". The Walker Patent neither teaches nor suggests that the treatment step is a chemical modification of the contaminating nucleic acids, which renders them removable from the sample, in contrast to the independent claims of the present application set forth above.

Primers are mentioned in the Walker patent on page 2, line 54 to page 4, line 2. The primers are merely discussed with reference to known amplification techniques. The use of the primers relates to known methods of amplifying the target DNA. In fact, on page 4, lines 16-21, the Walker patent teaches that the amplicons are preferably cleaved by the exonuclease. It further states on page 4, lines 22-26 that "...single stranded exonuclease can be used in the absence of primers. This is particularly advantageous due to the fact that double stranded exonuclease require the presence of primers during the exonuclease digestion step in order to provide double stranded ends on the amplicons." It is clear from a reading of the Walker patent, that it certainly does not teach a method

comprising the step of chemically treating a sample, wherein the chemical treatment results in the preferential chemical modification of the contaminating nucleic acid.

The examiner further argues that the Walker patent teaches the removal of the amplicons from the sample. However, the Walker patent actually teaches that the amplicons are degraded by the exonuclease. There is no suggestion that the contaminating nucleic acids are chemically modified in any way. For example, on page 2, lines 27-28, the Walker patent teaches that the nucleic acid sample and the single stranded exonuclease are incubated together for a sufficient time to degrade the amplicons. If the amplicons are degraded, then the primer/probe cannot bind to the amplicons. This is supported by the Examples of the present invention, which show that the aim of the method of the present invention is to completely degrade the amplicons, not render them removable from the sample using a primer or probe.

Further support is found in Examples 2 to 6 of the present application, which describe the decontamination of different nucleic acid samples which contain both genomic target DNA and amplicons (contaminating nucleic acids) using a variety of exonuclease enzymes and incubating these mixtures over several time periods. The samples are incubated with the exonuclease for a determined period of time. Example 2 shows the sample being incubated for 22 minutes with an exonuclease enzyme and then the enzyme was inactivated by heating for 10 minutes. After this step, the samples were subjected to a known amplification method, in this case SDA. Primers, shown in SEQ ID NO: 1 to 4, were used to amplify the target DNA. These primers are not associated with the amplicons and are not present as a treatment step before the sample is analyzed.

Example 2 further teaches that after the SDA reaction, (see page 5, lines 50-51) the SDA products were detected by hybridisation and extension of an amplicon specific radioactively labelled probe, shown as SEQ ID NO: 5. The Walker patent does not teach or suggest that the primer is

added to the nucleic acid sample prior to the amplification of the nucleic acid sample as a treatment step to render the amplicons or contaminating nucleic acids removable from the sample before they are the sample is analyzed.

In view of the foregoing, the Walker patent does not anticipate the new claims of the present invention.

Rejection under 35 U.S.C. § 102(b) - Satishchandran (U.S. Patent 6,168,918)

Claims 1, 2, 4-9, 12, 13 and 15 to 19 were rejected under 35 U.S.C. § 102(b) as being anticipated by Satishchandran (U.S. Patent 6,168,918). Claims 1, 2, 4-9, 12, 13 and 15 to 19 have been canceled in the present amendment, and therefore the rejection of these claims is moot. Applicants believe that the new claims are not anticipated by the Satishchandran patent as set forth below.

The Satishchandran patent is directed towards a method that tests whether or not foreign DNA is integrated into the chromosomal DNA of a eukaryote cell. It is not directed towards a method of chemically treating a sample to preferentially chemically modify the contaminating nucleic acids.

The Satishchandran patent describes a method of detecting the presence of a plasmid or viral DNA sequence integrated in a chromosomal DNA molecule of a eukaryotic cell in a sample that contains chromosomal DNA molecules of eukaryotic cells and non-integrated foreign DNA, wherein the foreign DNA has at least one DpnI restriction enzyme site as well as one or more non-DpnI restriction enzyme sites which can be cut by non-DpnI restriction enzymes. See Satishchandran, col. 2, lines 15 to 38. Thus, the method of the Satishchandran patent requires the essential step of

contaminating DNA to have at least one DpnI restriction site. See Satishchandran, col. 2, lines 28 to 33.

The method of the Satishchandran patent requires a complex number of steps to detect the presence of foreign DNA sequence integrated in a chromosomal DNA molecule. The method requires digestion of the sample, fractionation, an DpnI enzymic reaction, inactivation of the enzymic reaction, digestion of said DNA digestion resulting in fragments, amplification of fragments and detection of amplified fragments. See Satishchandran, col. 2, lines 44 to 62. More specifically, the method of Satishchandran comprises seven steps (a) to (g). Step (a) is the digestion of all DNA in the sample, including chromosomal DNA, foreign DNA sequences (which are integrated into the chromosomal DNA) and considered to be the target DNA, and foreign DNA that has not been integrated into the chromosomal DNA of the eukaryote cell. The digestion of the DNA is via a restriction enzyme. It should be noted that the target DNA in the Satishchandran patent is, in fact, the foreign DNA that has been integrated into the chromosomal DNA. In the Office Action, the Examiner incorrectly assumes that the integrated DNA is the contaminating nucleic acid and is required to be removed from the sample. However, the foreign DNA (integrated) is the nucleic acid sequence that is the subject of the amplification reaction and is not to be rendered removable from the sample before the sample is analyzed. The 'contaminating' nucleic acid is the bacterially derived plasmid or non-integrated foreign DNA (see for example column 11, line 65 to column 12, line 4). Here, the Satishchandran patent states that it is important to remove bacterially derived plasmid from the sample prior to amplification via PCR using a second round of enzyme digestion after the fractionation step (step b) of the method of the Satishchandran patent. Steps (a) to (e) neither teach nor suggest contacting the sample with a chemical to modify the nucleic acid.

The Examiner also argues that at Column 5, lines 29-67 and column 7, lines 28-67 Satishchandran teaches analyzing the nucleic acid sample. However, this section is irrelevant to the

present claims because the DNA discussed in the cited sections of the Satishchandran patent is not to be amplified. This particular section of Satishchandran, teaches step (e) of the method defined in claim 1 of Satishchandran. It describes the second use of an enzyme, in this case the use of DpnI to selectively cleave free plasmid DNA molecules in the presence of genomic DNA which might contain integrated plasmid sequences. This step is specifically designed to remove non-integrated DNA molecules by enzyme digestion. These DNA molecules (which might be considered by the Examiner to be 'contaminating' nucleic acids), are not the subject of chemical treatment.

The examiner has also cited to column 7, lines 29-67 of the Satishchandran patent as teaching the method of the present invention. This section of Satishchandran teaches the use of non-DpnI enzymes to degrade all DNA molecules in the sample. This is step (a) of the method of Satishchandran. At this section (see lines 37-38), the Satishchandran patent states that the plasmid sequences of interest (i.e. the target nucleic acids) are amplified using primers which amplify fragments that have an internal DpnI site. This amplification of plasmid sequences cannot be considered a chemical treatment step, as the purpose of treatment is to treat a sample prior to carrying out an amplification reaction. In contrast, new claims 43 and 50 of the present invention explicitly recite a treatment of a sample, whereby the nucleic acid sample is treated chemically to remove or inactivate contaminating nucleic acids.

At Column 8, lines 60-63, the Satishchandran patent teaches that the free plasmid DNA molecules (contaminating nucleic acids) are digested before carrying out PCR. In contrast, the sequences to be amplified in the method of the present invention are the target DNA and not the contaminating nucleic acids. This is further supported at Column 9, lines 4-8, where Satishchandran teaches that following DpnI digestion, only uncut DNA will yield amplification products.

The examiner further argues that the DpnI digested nucleic acids are contacted with primers

An early and favorable action on the merits is earnestly solicited.

Respectfully submitted,
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